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Genetic Variation in the *CYP2C* Monooxygenase Enzyme Subfamily Shows No Association With Longevity in a German Population

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Cytochrome P450 enzymes, especially the CYP2C subfamily, are involved in the generation of reactive oxygen species and are regarded as susceptibility factors for age-related diseases. Furthermore, the *CYP2C*-encoding genes are known to be highly polymorphic, with a number of variants leading to changes in enzyme activity. These observations prompted us to investigate whether allelic variation in the *CYP2C*-encoding genes was associated with human longevity. In a comprehensive haplotype tagging approach, we genotyped 56 single nucleotide polymorphisms located in the *CYP2C* gene family (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*) in our extensive collection of 1,384 long-lived individuals (centenarians and nonagenarians) and 945 younger controls. None of the tested single nucleotide polymorphisms showed a significant association with the longevity phenotype at the allele, genotype, or haplotype level. These results suggest that there is no notable influence of sequence variation in the *CYP2C* genes on longevity in the examined German population.

Key Words: Genetic association—Centenarians—Ageing—Cytochrome P450—CYP2C enzymes.

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LONGEVITY in humans is considered a multifactorial trait to which various genetic and environmental factors are likely to contribute. About 30% of the variation in adult life span is attributable to genetic parameters that show their strongest effect later in life (>60 years of age) (1–6). Epidemiological studies have revealed that people who survive to an exceptional old age (ie, ≥95 years) have often avoided or survived age-associated diseases. Hence, these long-lived individuals (LLI) show a favorable course of the ageing process and offer the unique opportunity to explore the genetic basis of the "healthy ageing" phenotype (7,8). It has been suggested that the genetic composition of the LLI differs from that of average-lived individuals in the following regards: (i) LLI are enriched for advantageous variants in so-called "longevity-enabling genes" and/or (ii) their genetic constitution shows a depletion of risk alleles for age-related diseases (9,10).

Cytochrome P450 enzymes (CYPs) are monooxygenases that are commonly known as important drug-metabolizing enzymes (11). They are also regarded as susceptibility factors for age-related cardiovascular diseases that represent the leading cause of death worldwide (12–14). Furthermore, CYP enzymes, particularly the CYP2C isoenzyme subfamily, are involved in the generation of reactive oxygen species (ROS) (15). Already more than 50 years ago, the accumulation of ROS was suggested to cause changes in physical or cognitive functions with ageing (16). To date, findings in the area of longevity research support a role of ROS and oxidative damage in age-related cellular decline ((17) and reviewed in (18)) and the development of age-related diseases (19).

In humans, the CYP2 enzyme subfamily C consists of four genes (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*) that are located next to each other on chromosome 10q (Figure 1). These enzymes are mainly expressed in human liver (20) but are also expressed in various other tissues, including the cardiovascular system, where they are involved in the modulation of vascular homeostasis by metabolizing endogenous regulators of vascular tone (21). Consequently, CYP2C inhibition has been reported to reduce ischemia–reperfusion injury in myocardial tissue (22–24). Furthermore, the *CYP2C*-encoding genes are also known to be highly polymorphic. Some of these variants

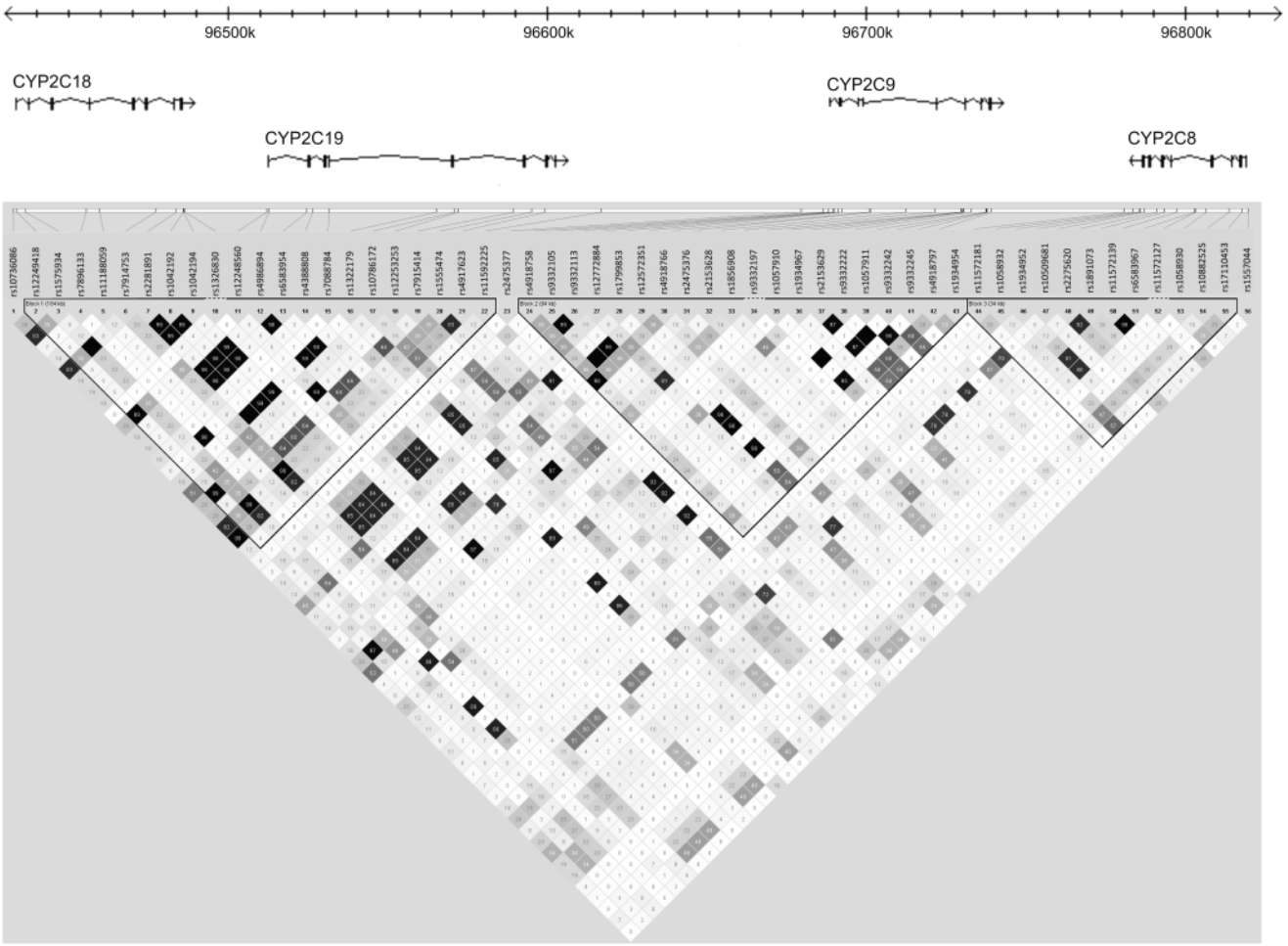

Figure 1. *CYP2C* gene region on chromosome 10. The physical position (in kilobases) of all 56 genotyped single nucleotide polymorphisms refers to the Genome Reference Consortium Human genome build 37. A schematic representation of the gene structures for *CYP2C18*, *CYP2C19*, *CYP2C9*, and *CYP2C8* is shown. The linkage disequilibrium (LD) plot of the locus is based on the measure r^2 and was generated with Haploview 3.32 using the data of the whole German case–control sample.

lead to a markedly reduced or no enzyme activity, whereas other alleles induce an increased activity or expression. In the context of drug metabolism, the variants *CYP2C9*2* (rs1799853) and *2C9*3* (rs1057910) associated with reduced enzyme activity are both known to be of particular clinical relevance. Recently, the U.S. federal drug agency (Food and Drug Administration) has encouraged prospective *CYP2C9* genotyping as a clinical tool to allow for individualized dose adjustment of the oral anticoagulant warfarin that is metabolized by *CYP2C9* (25).

As CYP2C enzymes also play an important role in the generation of ROS and are regarded as susceptibility factors for age-related diseases (15), they appear to be attractive candidates to be studied in the context of human longevity. Here, we performed a comprehensive fine mapping of the four *CYP2C* genes by testing altogether 56 single nucleotide polymorphisms (SNPs) in our extensive collection of 1,384 LLI (centenarians and nonagenarians) and 945 appropriately matched younger controls.

Methods

Participants

The LLI sample comprised 1,384 unrelated German study participants of exceptional age (age range: 95–109 years, mean: 98.8 years), including 616 centenarians (mean age: 101.3 years). The gender ratio was 73% females versus 27% males. The 945 German control participants were between 60 and 75 years of age (mean age: 66.9 years) and matched the LLI by ancestry, gender, and geographical origin within the country. A detailed description of the samples and the recruitment procedure is given elsewhere (26). All participants gave informed written consent prior to participation. The study was approved by the Ethics Committee of the University Hospital Schleswig-Holstein in Kiel.

SNP Genotyping

DNA samples from LLI and control participants were analyzed for 56 SNPs in the *CYP2C* gene region (Figure 1, Table 1) by using the SNPlex Genotyping System (Applied Biosystems, Foster City, CA) (27). The complete marker set consists of (i) a maximally informative panel of SNPs, selected through a haplotype tagging approach (based on the HapMap genotypes of Europeans with the pairwise tagging option; pairwise $r^2 \geq .8$; $p_{HWE} > .001$) to ensure that most of the allelic variation of the genomic regions was captured and the common haplotypes (≥2%) were represented; (ii) potentially functional SNPs that are located in exons, exon–intron boundaries, promoter regions, and 5′ and 3′ untranslated regions; and (iii) polymorphisms that have already been proven to be functionally relevant in the context of CYP2C enzyme activity (28–30). Of the 56 analyzed markers, 14 are located in the *CYP2C8* gene, 20 SNPs in *CYP2C9*, 10 SNPs in *CYP2C18*, and 12 SNPS in *CYP2C19* (Figure 1, Table 1).

Statistical Analysis

Allele-based single marker case–control analyses (CCA) were performed with χ^2 statistics and the appropriate degrees of freedom using the open-source analysis toolset PLINK v.1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). *p* values smaller than .05 were considered nominally statistically significant, and Bonferroni correction for 56 tests was applied to the single-point results: Of the 56 tested markers, 28 were in strong linkage disequilibrium with each other (pairwise $r^2 > .8$; calculated with the Haploview tagger pairwise option) so that the number of markers considered for the Bonferroni correction could be reduced to 28. As two case–control analyses (whole sample and centenarians) were performed, altogether 56 tests need to be taken into account. The software programme Haploview version 4.1 (http://www.broad.mit.edu/mpg/haploview/) was used to assess all polymorphisms for significant deviation from the Hardy–Weinberg equilibrium (HWE), to calculate linkage disequilibrium (r^2) between each marker pair, and to conduct haplotype association analyses in blocks (31).

Results

The whole sample of 1,384 German LLI, a subset of 616 centenarians, and a control group of 945 younger individuals were subjected to a gender-matched case–control analysis of 56 SNPs located in the *CYP2C* genes (*CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*). All SNPs were found to be in HWE ($p > .001$). Only one nominally significant association signal (rs11188059; $p_{CCA} = .04$) was observed in the analysis of the centenarian sample (Table 1) that did not pass correction for multiple testing (Bonferroni-adjusted $p_{CCA} = 1$, assuming 56 tests; see "Participants and Methods" and "Statistical Analysis"). In the entire longevity sample (1,384 LLI and 945 controls), none of the tested SNPs showed a significant association, even without consideration of multiple testing (data not shown).

The 56 SNPs form three haplotype blocks (Figure 1). Block 1 comprises 20 markers, Block 2 comprises 19 markers, and Block 3 comprises 11 markers (Figure 1), which define eight common haplotypes (each present at a frequency of at least 2% in the population) for each block. None of the observed haplotypes differed significantly in frequency between cases and controls (data not shown).

Discussion

Cytochrome P450 enzymes, especially the *CYP2C* isoforms, are involved in the generation of ROS (15). They are expressed in tissues of the cardiovascular system and are considered susceptibility factors for age-related diseases (15). Furthermore, the *CYP2C*-encoding genes are known to be highly polymorphic, with a number of variants leading to changes in enzyme activity.

These observations prompted us to investigate whether allelic variation in the *CYP2C*-encoding genes was associated with human longevity. Altogether, we genotyped 56 markers in our extensive DNA collection of more than 2,300 LLI and controls. None of the tested SNPs or haplotypes showed a statistically significant association with longevity, neither in the whole sample nor in the centenarian subset.

Candidate gene association studies have emerged as powerful tools in longevity research (32–47). So far, two longevity relevant genes (*APOE* and *FOXO3A*) have been confirmed in many different populations (32,35–37,39,48–55). Because *APOE* and *FOXO3A* have been identified by candidate gene association studies, it seems that this method is still relevant for human longevity research, even in the era of genome-wide association studies. Although genome-wide association studies offer the advantage of detecting new longevity genes without a priori hypothesis, the power of hypothesis-driven candidate approaches is much higher than that of genome-wide association studies where millions of SNPs are tested, and multiple comparisons have to be taken into consideration as an essential part of determining statistical significance (56). Hence, so far it has been difficult to detect new longevity variants by genome-wide studies, and apart from the *APOE* locus, none of the reported genome-wide association studies signals achieved conventional levels of statistical significance (51,57,58). Altogether, candidate gene association studies still play an important role for the identification of longevity loci.

With the applied approach, we are likely to have captured all common variation present in the analyzed samples for the *CYP2C* gene region. Acknowledging that the selected markers might be insufficient to tag the genetic variation comprehensively, we cannot rule out the presence of rare polymorphisms that could influence longevity. However, with the consideration of haplotype differences, if present,

Table 1. Association Statistics for 56 SNPs Located in the *CYP2C* Gene Region (for the centenarian subset)

Gene	SNP	Position on CHR 10 (GRCh37/hg19)	Min AF Cases	Min AF Controls	p_{CCA}	Bonferroni-Adjusted p_{CCA}
CYP2C18	rs10736086	96441650	.489	.503	.45	1
	rs12249418	96442917	.242	.215	.08	1
	rs1575934	96445609	.464	.448	.36	1
	rs7896133	96464730	.059	.069	.24	1
	rs11188059	**96468899**	**.106**	**.131**	**.04**	**1**
	rs7914753	96486504	.464	.448	.36	1
	rs2281891	96493058	.164	.164	.99	1
	rs1042192	96495284	.166	.167	.97	1
	rs1042194	96495484	.164	.163	.92	1
	rs1326830	96495793	.008	.012	.28	1
CYP2C19	rs12248560	96521657	.241	.216	.11	1
	rs4986894	96522365	.164	.165	.95	1
	rs6583954	96534263	.166	.166	.99	1
	rs4388808	96536056	.158	.168	.47	1
	rs7088784	96541373	.059	.068	.31	1
	rs1322179	96575242	.164	.164	.99	1
	rs10786172	96581094	.334	.344	.54	1
	rs12253253	96582156	.241	.213	.07	1
	rs7915414	96599510	.223	.232	.54	1
	rs1555474	96605327	.464	.450	.44	1
	rs4917623	96609568	.491	.502	.55	1
	rs11592225	96627191	.143	.136	.59	1
CYP2C9	rs2475377	96690371	.044	.049	.53	1
	rs4918758	96697252	.379	.381	.93	1
	rs9332105	96698925	.186	.181	.71	1
	rs9332113	96700402	.187	.181	.68	1
	rs12772884	96700630	.386	.416	.10	1
	rs1799853	96702047	.122	.125	.82	1
	rs12572351	96703220	.186	.180	.65	1
	rs4918766	96711884	.380	.377	.87	1
	rs2475376	96712400	.148	.149	.92	1
	rs2153628	96723424	.236	.212	.11	1
	rs1856908	96732731	.339	.362	.20	1
	rs9332197	96740908	.038	.049	.14	1
	rs1057910	96741053	.058	.065	.44	1
	rs1934967	96741426	.191	.211	.17	1
	rs2153629	96741795	.135	.129	.66	1
	rs9332222	96744064	.134	.125	.46	1
	rs1057911	96748737	.058	.066	.42	1
	rs9332242	96748893	.134	.125	.46	1
	rs9332245	96749181	.063	.068	.52	1
	rs4918797	96750251	.196	.196	.98	1
CYP2C8	rs1934954	96792202	.090	.079	.27	1
	rs11572181	96795046	.061	.052	.29	1
	rs1058932	96796861	.201	.197	.81	1
	rs1934952	96797500	.344	.346	.92	1
	rs10509681	96798749	.118	.107	.36	1
	rs2275620	96802598	.345	.372	.12	1
	rs1891073	96804911	.305	.327	.19	1
	rs11572139	96808886	.302	.296	.70	1
	rs6583967	96814475	.301	.294	.70	1
	rs11572127	96814689	.043	.049	.45	1
	rs1058930	96818119	.052	.059	.41	1
	rs10882525	96825332	.353	.333	.24	1
	rs17110453	96829529	.146	.150	.73	1
	rs1557044	96831389	.136	.138	.88	1

Notes: Centenarian subset: 616 German centenarians = 100–109 years; 945 younger controls = 60–75 years; CHR 10 = chromosome 10; GRCh37 = Genome Reference Consortium Human genome build 37; hg19 = release of the February 2009 human genome browser, UCSC version hg19; Min AF = minor allele frequency; $p_{CCA} = p$ value obtained from an allele-based case-control comparison using a χ^2 test with 1 *df*; SNP = single nucleotide polymorphism. Bold indicates SNP showed a nominally significant association signal in the analysis of the centenarian sample but did not pass correction for multiple testing.

the effect of rare variants should be statistically detectable as these effects ought to be carried by one of the common background haplotypes (59). Furthermore, common variants can act as significant modifiers of the effects of rare variants (60). Thus, rare variants that are functionally relevant are often identified by common variant associations (61–64). Altogether, it seems unlikely that we have missed such rare variant effects in our comprehensive approach. The possibility that the negative association finding is due to population stratification in our samples is also rather improbable because the validity and efficacy of our large and well-characterized study population for genetic longevity research have already been shown with the identification and validation of previous association findings (26,32–34). Overall, our results suggest that there is no noteworthy influence of sequence variation in the *CYP2C* genes on human longevity in Germans.

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